

CLAIMS

1. A method for enriching the GC base pair content of a DNA molecule the method comprising the steps of (a) providing a DNA template molecule in which at least some of the A residues are base paired with U residues and (b) replicating the DNA template molecule provided in step (a) under conditions in the replication reaction medium in which at least some of the U residues base pair with a G residue.

2. A method according to Claim 1 wherein the DNA template molecule in (a) is produced by replicating a first template DNA molecule in the presence of dUTP so that at least some of the T residues of the first template are replaced by U residues to form a second template molecule.

3. A method according to Claims 1 and 2 comprising the steps of (1) providing a first template DNA molecule, (2) replicating the first template DNA molecule in the presence of dUTP so that at least some of the T residues of the first template are replaced by U residues to form a second template molecule and (3) replicating the DNA template molecule produced in step (2) under conditions in the replication reaction medium in which at least some of the U residues base pair with a G residue.

4. A method according to any one of the preceding claims wherein the conditions in which at least some of the U residues base pair with a G residue are provided by an agent which is able to increase the polarity of the replication reaction medium and/or act as a local dehydrating agent.

5. A method according to Claim 4 wherein an excess of dGTP over dATP or dCTP or dTTP is present in the reaction medium during replication.

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6. A method according to Claim 4 or 5 wherein the agent which is able to increase the polarity of the reaction medium and/or act as a local dehydrating agent is polyethylene glycol (PEG).

10 7. A method according to Claim 6 wherein the PEG is PEG300-PEG8000.

8. A method according to any of the preceding claims wherein the DNA replication is by a polymerase chain reaction.

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9. A method according to any one of the preceding claims wherein the DNA molecule whose GC base pair content is to be enriched comprises all or part of a natural gene or cDNA.

20 10. A method according to Claim 9 wherein the gene or cDNA has a GC base pair content lower than 50%.

11. A method according to Claim 9 or 10 wherein the gene or cDNA encodes a polypeptide of interest.

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12. A method according to Claim 10 wherein the polypeptide is any of an enzyme, an antibody chain or an antigen.

13. A method according to any one of the preceding claims comprising the further step of cloning the DNA molecule whose GC base pair content has been enriched.
- 5 14. A method according to Claim 13 wherein the DNA molecule whose GC base pair content has been enriched is cloned into an expression vector.
- 10 15. A method according to any one of the preceding claims comprising the further step of sequencing the DNA molecule whose GC base pair content has been enriched.
- 15 16. A method according to Claim 15 comprising the further step of selecting those of the DNA molecules whose GC base pair content has been enriched wherein the coding sense has been retained.
- 20 17. A method according to Claim 15 comprising the further step of selecting those of the DNA molecules whose GC base pair content has been enriched wherein coding sense has been altered.
- 25 18. A method according to Claim 11 comprising the further step of expressing a polypeptide from the gene or cDNA whose GC base pair content has been enriched.
- 30 19. A DNA molecule enriched for GC base pair content prepared by the method of any of Claims 1 to 14.
20. A method for making a mutant polypeptide with altered properties compared to the polypeptide encoded by a given DNA molecule, the method comprising (a) enriching the GC base pair content of the

DNA molecule according to the method of any of Claims 1 to 12, (b) expressing the polypeptide encoded by the DNA molecule whose GC base pair content has been enriched in step (a), and (c) selecting a polypeptide with altered properties.

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21. A method according to Claim 20 wherein the polypeptide is any of an enzyme, an antibody chain or an antigen.

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22. A method according to Claim 21 wherein the polypeptide is an enzyme which has been selected in step (c) for improved catalytic properties.

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23. A method according to Claim 22 wherein the enzyme is encoded by the *albD* gene of *Pantoea dispersa*.

24. A mutant polypeptide prepared by the method of any of Claims 20 to 23.

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25. A mutant AlbD polypeptide wherein Ser40 has been replaced by another amino acid residue.

26. A mutant AlbD polypeptide according to Claim 25 wherein Ser40 has been replaced with Gly.

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27. A mutant AlbD polypeptide according to Claim 25 or 26 wherein Glu25 has been replaced by Arg, Lys27 has been replaced by Glu and Ser40 has been replaced by Gly.

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28. A polynucleotide encoding the mutant AlbD polypeptide according to Claim 25.

29. An expression vector containing a polynucleotide according to Claim 28.
- 5 30. A transgenic plant containing a polynucleotide according to Claim 28.
- 10 31. A kit of parts for enriching the GC base pair content of a DNA molecule in a replication reaction medium comprising (a) dUTP and (b) an agent which is able to increase the polarity of the replication reaction medium and/or act as a local dehydrating agent.
- 15 32. A kit of parts according to Claim 31 wherein the agent which is able to increase the polarity of the replication reaction medium and/or act as a local dehydrating agent is a polyethylene glycol.
33. A kit of parts according to Claim 31 or 32 further comprising other reagents for carrying out a DNA amplification reaction.
- 20 34. Any novel method of enriching the GC base pair content of a DNA molecule for *in vitro* evolution as described herein.